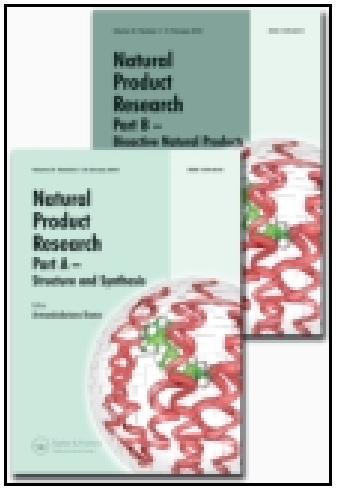

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

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Published online: 08 May 2014.

To cite this article: Jaggaiah N. Gorantla, Jamsheena Vellekkatt, Lekshmi R. Nath, Ruby John Anto & Ravi S. Lankalapalli (2014) Cytotoxicity studies of semi-synthetic derivatives of theveside derived from the aqueous extract of leaves of 'suicide tree' Cerbera odollam, Natural Product Research: Formerly Natural Product Letters, 28:18, 1507-1512, DOI: 10.1080/14786419.2014.913242

To link to this article: http://dx.doi.org/10.1080/14786419.2014.913242

Natural Product Research, 2014
Vol. 28, No. 18, 1507–1512, http://dx.doi.org/10.1080/14786419.2014.913242

SHORT COMMUNICATION

Cytotoxicity studies of semi-synthetic derivatives of theveside derived from the aqueous extract of leaves of 'suicide tree' *Cerbera odollam*

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(*Received 27 February 2014; final version received 4 April 2014*)

We report the isolation of two known iridoid glucosides theviridoside (**1**) and theveside (**2**) from the aqueous extract of leaves of *Cerbera odollam* and semi-synthetic derivatisation of theveside prepared in a single step under protection group-free conditions. Derivatives **2a**–**j** were evaluated for cytotoxicity towards five human cancer cell lines of different origins, namely SKBR3 (breast), HeLa (cervical), A375 (skin), HepG2 (liver) and HCT-116 (colon), and IC_{50} values were determined. Derivatives **2b** and **2h** exhibited moderate cytotoxicity against HCT-116 and A375 cell lines, respectively.

Keywords: *Cerbera odollam*; theviridoside; theveside; semi-synthetic derivatives; cytotoxicity

1. Introduction

Cerbera odollam, a mangrove plant, belongs to the poisonous Apocynaceae family found along coastal swamps, riverbanks and creeks in many Asian countries (Gaillard et al. 2004). *C. odollam* is also known as *Cerbera mangha* owing to its striking resemblance with mango tree. *C. odollam* is known for prevalence in self-poisoning cases from southern states of India, which is attributed to about 50% of the plant poisoning cases and 10% of the total poisoning cases in Kerala, India (Gaillard et al. 2004). The poisonous nature is attributed to cardiac glycosides known as cardenolides that are present in the latex throughout the plant and seed (Laphookhieo et al. 2004; Eddleston & Haggalla 2008). Cardiac glycosides are the predominant constituents from the genus *Cerbera*, and other chief constituents include lignans, terpenoids, flavonoids and progesterones (Shen et al. 2007).

In general, pursuit towards phytochemical investigations involves isolation of compounds from organic solvent extracts, namely *n*-hexane, ethyl acetate, chloroform and methanol. The aqueous extracts are usually discarded or less investigated owing to the challenges in purification and most importantly in characterisation and identification, albeit medicinal plants used in ayurvedic medicine typically involve aqueous preparations. In this study, we explored the phytochemical constituents of an aqueous extract from the leaves of *C. odollam* and isolated two known iridoid glucosides, theviridoside (**1**) and theveside (**2**) (Table 1). The most polar major constituent isolated was treated with acetic anhydride, and the peracetylated derivative was found to be sucrose. Theveside (**2**) with a free carboxylic acid offers an opportunity to build

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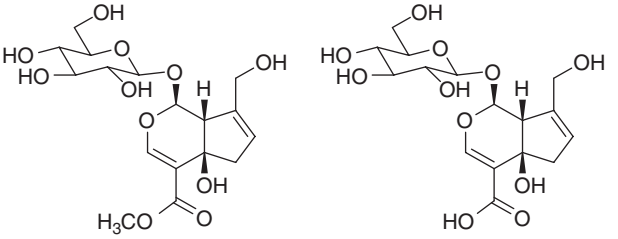
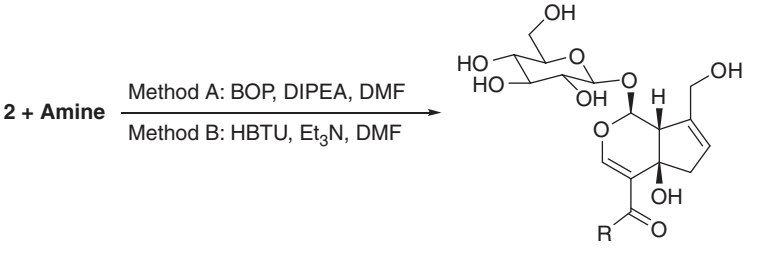
Table 1. Structures of theviridoside (**1**), theveside (**2**) and semi-synthetic derivatives.





Entry[a]	R	Yield (%)[b]
2a	4-Me-C_6H_4NH	50
2b	4-MeO-C_6H_4NH	52
2c	4-Cl-$C_6H_4CH_2NH$	80
2d	$C_6H_4CH_2NH$	55
2e	*N*,*N*-Allyl, methyl-N	38
2f	Butyl-NH	69
2g	*t*-Butyl-NH	68
2h	Cyclohexyl-NH	48
2i	1-Phenylethyl-1-NH	73
2j	*N*,*N*-Dimethyl-N	48

Abbreviations: BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylethylamine.
[a] Compounds **2a**, **2b** and **2j** were synthesised by using Method A. Compounds **2c**–**2i** were synthesised by using Method B.
[b] Isolated yields.

diversity along the skeleton to produce a library of pharmacologically relevant semi-synthetic derivatives. Accordingly, 10 amide derivatives **2a**–**j** (Table 1) were synthesised in a single step and screened for cytotoxicity towards five human cancer cell lines. Theveside displayed no significant cytotoxicity in any of the cancer cell lines tested, while two of its semi-synthetic derivatives **2b** and **2h** exhibited moderate cytotoxicity against HCT 116 and A375 cell lines, respectively.

2. Results and discussion

2.1. *Isolation of compounds*

The leaf material was dried in a hot air oven between 45 to 50°C and then ground to powder form. To 50 g of the ground plant material, distilled water (1 L) was added and heated between 90 to 100°C for 6 h. After attaining room temperature, the mixture was left to stand overnight and then filtered. The crude aqueous filtrate was subjected to lyophilisation which afforded 18 g of

the dried material. A 100.0 mg of lyophilised aqueous extract was purified by reverse-phase preparative HPLC, for quantification, using the following gradient program: solvent A, H_2O; solvent B, MeOH; linear gradient 0 min 10% B, 35 min 50% B, 40 min 80% B, 45 min 100% B; detection by charring TLC after spraying with 15% sulphuric acid. Three major products were isolated and lyophilised to obtain sucrose (41.0 mg), iridoid glucosides theviridoside **1** (4.0 mg) and theveside **2** (7.4 mg) (Table 1). The NMR results were in close agreement with the literature (Martin et al. 2007). Iridoids are a group of cyclopenta[*c*]pyran monoterpenoids the name of which was derived from Australian ants, *Iridomyrmex*, which use these molecules for defence mechanisms (El-Naggar & Beal 1980; Boros & Stermitz 1991). Iridoids exhibit a wide range of bioactivities (Tundis et al. 2008), two such iridoid glucosides aucubin and geniposide exert antitumoral activity by inhibition of topoisomerase I (Galvez et al. 2005).

2.2. *Cytotoxicity studies*

The *in vitro* cytotoxicity reports of iridoid glucosides isolated from alcoholic and aqueous extracts exhibited a good to moderate activity with concentrations in the range of μM against different human cancer cell lines (Li et al. 2012; Saracoglu & Harput 2012; Rana et al. 2014). Semi-synthetic derivatives of iridoid glucosides were also reported for *in vitro* cytotoxicity

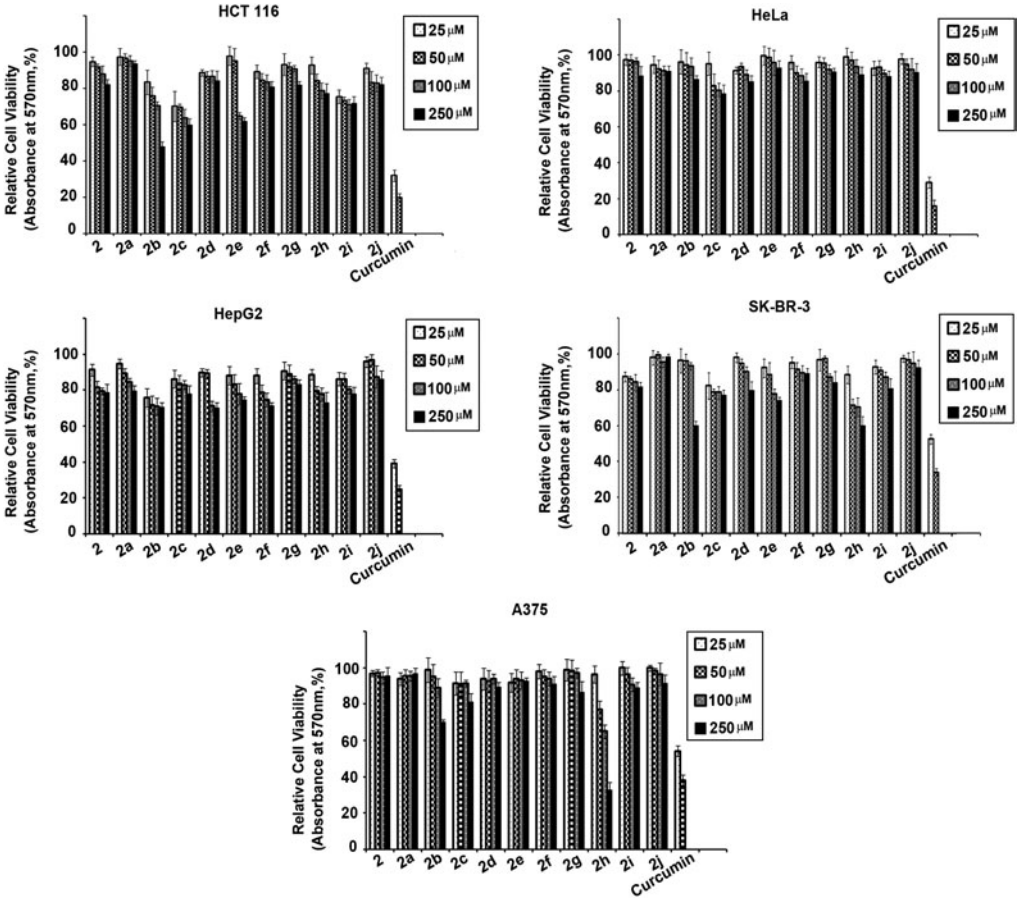


Figure 1. Dose-dependent effect of theveside (**2**) and its semi-synthetic derivatives **2a**–**j** on five cancer cell lines.

studies, which exhibited a better activity than their parent compound (Mouries et al. 2005; Rakotondramasy et al. 2010). As theveside **2** possesses a carboxylic acid which can be derivatised to amides, we have synthesised 10 amide derivatives (**2a**–**j**, Table 1) in a library-based approach in a single step with different aromatic and aliphatic amines. Standard peptide bond coupling agents such as (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate and *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate were used for the synthesis under hydroxyl group protection free conditions. In order to determine the cytotoxicity of theveside **2** and its derivatives **2a**–**j** against various cancer cells, dose-dependent effect of the compounds on a panel of five cancer cell lines were determined using the MTT cell viability assay at concentrations ranging from 25 to 200 μM after incubation for 72 h (Figure 1). A dose-dependent effect on cytotoxicity of the derivatives was clearly evident from the cell lines with increase of concentration. The IC_{50} value of compound **2h** on A375 cells was approximately 150 μM, and for compound **2b** on HCT-116 was approximately 190 μM (Table S1, Supplementary material). IC_{50} values of the remaining compounds were greater than 200 μM. The results clearly indicate that theveside **2** and its derivatives **2a**–**j** did not affect cell viability of the cervical (HeLa), liver (HepG2) and breast (SKBR3) cancer cells tested. From a structure–activity standpoint on A375 cells, compound **2h** with a cyclohexane ring is more cytotoxic among the derivatives containing aromatic, aliphatic acyclic and aliphatic cyclic amides. The MTT results also show that theveside possesses no significant toxicity against various cancer cell lines, while compound **2h** with moderate cytotoxicity indicates that a library of cyclic aliphatic amides as well as fused and branched bicyclic amides of theveside could exhibit better cytotoxicity.

3. Experimental

3.1. *Plant material*

Leaves of *C. odollam* Gaertn. were collected from Veli lake side, Thiruvananthapuram, Kerala, India, and were identified by E.S. Santhoshkumar, Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI), Thiruvananthapuram, Kerala. A voucher specimen (No. 76807) has been deposited in the JNTBGRI Herbarium.

3.2. *Cancer cells*

Five human cancer cell lines of various origins (SKBR3: breast, HeLa: cervix, A375: skin, $HepG_2$: liver, HCT-116: colon) were procured from the National Centre for Cell Sciences, Pune, India. The cells were maintained in DMEM containing 10% FBS and antibiotics at 37°C in a CO_2 incubator.

3.3. *MTT assay*

Cytotoxicity of the synthesised compounds **2a**–**j** along with its parent compound theveside **2** was evaluated by standard MTT assay in five different human cancer cell lines after 72 h of treatment, as described earlier (Bava et al. 2005). Cells (3000 cells/well) were seeded in 96-well plates and kept overnight at 37°C. The cells were then incubated with different concentrations (25, 50, 100 and 200 μM) of theveside **2** and its derivatives **2a**–**j**. Curcumin was used as the positive control. After 72 h of incubation, the percentage of viable cells was determined by MTT assay. Briefly, after the treatment period, the cells were washed with PBS after removing the media containing the compounds and then were incubated with fresh medium containing 20% MTT solution for 2 h. The incubation was continued for another 1 h after addition of extraction buffer (20% SDS in DMF) to dissolve the newly formed complex. Finally, the optical density

was measured at 570 nm using a plate reader (Bio-Rad, Hercules, CA, USA) and compared with that of untreated control. The data were subjected to linear regression analysis for calculation of IC_{50} concentrations. Data are average of three independent sets of experiments.

4. Conclusions

The aim of this study was to identify the phytochemical constituents of aqueous extract from the leaves of poisonous tree *C. odollam*. We have identified for the first time the presence of sucrose in large amounts in the leaves along with two known iridoid glucosides theveside **2** and theviridoside **1**. Theveside and its semi-synthetic derivatives **2a**–**j** were tested for *in vitro* cytotoxic activity against five cancer cell lines of different origins. Among the compounds screened, compound **2h** displayed the maximum cytotoxic activity in A375 cells (IC_{50} 150 μM) while most of the compounds did not affect cell viability of the cervical (HeLa), liver (HepG2) and breast (SKBR3) cancer cell lines. The reason behind this variation in sensitivity of the cancer cells towards the theveside derivatives is yet to be studied. However, we are trying to pursue the utility of these derivatives against other disease model cell-based assays.

Supplementary material

Supplementary material relating to this article is available online, including general procedures, NMR spectral data of theviridoside **1**, theveside **2** and synthesis of theveside derivatives **2a**–**j**. Cytotoxic activity (IC_{50}, μM) of theveside **2** and its derivatives **2a**–**j**. NMR and mass spectra of theviridoside **1** and theveside **2**.

Acknowledgement

This work was financially supported by the Council of Scientific and Industrial Research (CSIR), India, under the 12th Five-Year Plan (FYP) project NAPAHA.

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